

DOCKET NO.: WARF 0138 ((P98103 CIP) - 2 -

PATENT

separated from RNA molecules with which it would be associated in its natural state (i.e., in cells or tissues), such that it exists in a "substantially pure" form (the term "substantially pure" is defined below). Nucleic acid sequences and amino acid sequences can be compared using computer programs that align the similar sequences of the nucleic or amino acids thus define the differences. For purposes of this invention, the DNASTar program (DNASTar, Inc., Madison, Wisconsin) and the default parameters used by that program are the parameters intended to be used herein to compare sequence identity and similarity. Alternately, the Blastn and Blastp 2.0 programs provided by the National Center for Biotechnology Information (Altschul et al., 1990, J Mol Biol 215:403-410) using a gapped alignment with default parameters, may be used to determine the level of identity and similarity between nucleic acid sequences and amino acid sequences.

Please replace the paragraphs following the **Brief Description of the Drawings** at page 6, line 29 with the following rewritten paragraphs:

Figures 1 - 4. Amino acid sequence lineup of ATPAC deduced amino acid sequence and the amino acid sequences of related mammalian and plant genes. The lineup shows the ATPAC deduced amino acid sequence (SEQ ID NO:2) compared with (1) hmdr1 (SEQ ID NO:3); (2) mmdr1 (SEQ ID NO: 4); (3) hmdr3 (SEQ ID NO:5); (4) mmdr2 (SEQ ID NO:6); (5) atpgp1 (SEQ ID NO:7); and (6) atpgp2 (SEQ ID NO:8). A consensus sequence (SEQ ID NO: 9) is also shown. **Figure 1.** Amino acids corresponding to 1-440 of the consensus sequence. **Figure 2.** Amino acids corresponding to 441-880 of the consensus sequence. **Figure 3.** Amino acids corresponding to 881-1210 of the consensus sequence. **Figure 4.** Amino acids corresponding to 1211-1325 of the consensus sequence.

DOCKET NO.: WARF 0138 ((P98103 CIP) - 3 -**PATENT**

Figure 5. Graph depicting the effect of rhodamine 6G on the growth rate of cells transformed with and expressing ATPAC as compared with control cells not containing ATPAC.

Figure 6. Restriction map of genomic clone of ATPAC, SEQ ID NO:10.

Figure 7. Restriction map of cDNA clone of ATPAC, SEQ ID NO:1

Please replace the paragraph beginning at page 13, line 11 with the following rewritten paragraph:

A genomic clone of ATPAC (SEQ ID NO:10) has also been isolated from a bacterial artificial chromosome (BAC) library of the Arabidopsis genome (BAC clone IGF F3J22, obtained from the Arabidopsis stock center, Ohio State University). A 7 kb fragment containing part of ATPAC and additional 5' regulatory sequences was subcloned into a plasmid vector (pBluescript). A restriction map of ATPAC is found in Fig. 6. The corresponding cDNA clone of ATPAC is found in SEQ ID NO:1 and its restriction map is Fig. 7.

Please replace the two paragraphs beginning at page 14, line 27 with the following rewritten paragraphs:

In other preferred embodiments, the nucleic acids have a restriction digest map that is identical for at least 3 enzymes (more preferably 6 enzymes and most preferably 9 enzymes) to the maps shown in Figs. 6 or 7. In another preferred embodiment, the nucleic acids have a restriction digest map identical to those shown in Fig. 6 for enzymes XhoI, XcmI and SpeI

DOCKET NO.: WARF 0138 ((P98103 CIP) - 4 - PATENT

(preferably additionally SacI, PacI and BsaI, and most preferably additionally AclI, BanI and SnaBI).

In another preferred embodiment, the nucleic acids have a restriction digest map identical to those shown in Fig. 7 for enzymes XbaI, TatI and NciI (preferably additionally DraI, BsmI and BclI, and most preferably additionally AccI, BsgI and TliI). The nucleic acids of the invention are at least 20 nucleic acids in length (preferably at least 50 nucleic acids and most preferably at least 100 nucleic acids).

Please replace the paragraph beginning at page 22, line 13 with the following rewritten paragraphs:

In accordance with the present invention, nucleic acids having the appropriate level sequence homology with part or all the coding regions of SEQ ID NO:1 or SEQ ID NO:10 may be identified by using hybridization and washing conditions of appropriate stringency. For example, hybridizations may be performed, according to the method of Sambrook et al., using a hybridization solution comprising: 5X SSC, 5X Denhardt's reagent, 1.0% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA, 0.05% sodium pyrophosphate and up to 50% formamide. Hybridization is carried out at 37-42 C for at least six hours. Following hybridization, filters are washed as follows: (1) 5 minutes at room temperature in 2X SSC and 1% SDS; (2) 15 minutes at room temperature in 2X SSC and 0.1% SDS; (3) 30 minutes-1 hour at 37 C in 2X SSC and 0.1% SDS; (4) 2 hours at 45-55 C in 2X SSC and 0.1% SDS, changing the solution every 30 minutes.

One common formula for calculating the stringency conditions required to achieve hybridization between nucleic acid molecules of a specified sequence homology (Sambrook

DOCKET NO.: WARF 0138 ((P98103 CIP) - 5 -**PATENT**

et al., 1989):

Please replace the paragraph beginning at page 23, line 13 with the following rewritten paragraph:

The stringency of the hybridization and wash depend primarily on the salt concentration and temperature of the solutions. In general, to maximize the rate of annealing of the probe with its target, the hybridization is usually carried out at salt and temperature conditions that are 20-25 C below the calculated T_m of the of the hybrid. Wash conditions should be as stringent as possible for the degree of identity of the probe for the target. In general, wash conditions are selected to be approximately 12-20 C below the T_m of the hybrid. In regards to the nucleic acids of the current invention, a moderate stringency hybridization is defined as hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 $\mu\text{g/ml}$ denatured salmon sperm DNA at 42 C, and wash in 2X SSC and 0.5% SDS at 55 C for 15 minutes. A high stringency hybridization is defined as hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 $\mu\text{g/ml}$ denatured salmon sperm DNA at 42 C, and wash in 1X SSC and 0.5% SDS at 65 C for 15 minutes. A very high stringency hybridization is defined as hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 $\mu\text{g/ml}$ denatured salmon sperm DNA at 42 C, and wash in 0.1X SSC and 0.5% SDS at 65 C for 15 minutes.

Please replace the paragraph beginning at page 34, line 1 with the following rewritten paragraph:

DOCKET NO.: WARF 0138 ((P98103 CIP)) - 6 - PATENT

The plPAC of the present invention was identified by its up-regulation in response to a chloride ion channel blocker. *Brassica napus* plants were grown either in the presence or absence of 20 μ M 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB). After five days, the roots of the seedlings were harvested and total RNA was extracted separately from the treated and untreated plants. From the total RNA preparations, poly (A)+ RNA was isolated and used as the starting material to create a cDNA subtraction library, using the CLONTECH PCR-SELECTTM cDNA Subtraction Kit and accompanying instructions (CLONTECH Laboratories, Inc., Palo Alto, CA).

Please replace the paragraph beginning at page 34, line 22 with the following rewritten paragraph:

The 3.76 kb cDNA clone encodes a polypeptide 1,254 amino acids in length. The deduced amino acid sequence encoded by SEQ ID NO:1 is shown in Figures 1-4 as "atpac" (SEQ ID NO:2), in a lineup with the following sequences: (1) hmdr1 (SEQ ID NO:3); (2) mmdr1 (SEQ ID NO:4); (3) hmdr3 (SEQ ID NO:5); (4) mmdr2 (SEQ ID NO:6); (5) atpgp1 (SEQ ID NO:7); and (6) atpgp2 (SEQ ID NO:8). A consensus sequence (SEQ ID NO:9) is also shown.

Please replace the paragraph beginning at page 35, line 21 with the following rewritten paragraph:

The compound Rhodamine 6G is a well known substrate of mammalian p-glycoproteins (Kolaczowski et al., J. Biol. Chem. 271: 31543-31548, 1996). The ability of a